

Research Article**Study of Soil Cyanobacteria to Evaluate Metabolite Production during various incubations in their Culture Filtrate**

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Abstract: Cyanobacteria are a remarkable group of photosynthetic prokaryotes. It survives in wide range habitat in soil and also morphologically various from unicellular to filamentous thalli. Blue green algae produced many metabolites including amino acids, proteins, vitamins and plant growth regulators i.e. auxins, gibberellins and abscisic acids. The present study is to estimate biomass, total protein and plant growth hormone (Indole-3-acetic acid) in their culture filtrate during various incubation times using BG-11 broth. Protein and Indole-3-acetic acid was estimated from culture filtrate by standard protocols. By observing it was found that amount of biomass, protein and IAA was increased with incubation time and shows maximum concentration as 300 mg, 750 µg/ml and 4.0 mg/L after 30 days respectively by *Nostoc* spp.

Keywords: Cyanobacteria, Biomass, Protein, Indole-3-acetic acid, Incubation time.

INTRODUCTION

Blue green algae are the most diverse and dominant photosynthetic prokaryotes found in soil that secrets carbon and nitrogen compounds in soil [10]. The plant growth regulators e.g. gibberellin, auxin, cytokinin, ethylene and abscisic acid have been detected in cyanobacteria [14, 15]. Cyanobacterial biomass extracts of *Plectonema* spp. stimulated somatic embryogenesis and development in sandalwood, *Santalum album* [2].

Cyanobacterial strains forming mucilaginous colonies which help to dominance of this organism in soil especially *Nostoc* spp. [15]. Comparative biomass production and nitrogen fixation has been examined in large number of cyanobacterial strains including members of Rivulariaceae such as *Gloeotrichia* and *Calothrix* and reported that *Gloeotrichia* spp. exhibited higher dry weight and *Calothrix* higher nitrogen fixation [6, 19].

Cyanobacteria characteristically liberate substantial quantities of extracellular nitrogenous compounds into the medium and also produced combined nitrogen in soluble form by nitrogen fixing BGA [21, 22]. They add organic matter and secrete growth promoting substances like vitamins, auxin and amino acids [17, 20].

The cyanobacteria are a remarkable group of prokaryotes which can survive independently and also in association with other organisms such as lichen, liverwort and mosses [3]. They secrets commonly plant growth regulators (auxins, gibberellins, and ethylene), amino acids, protein and vitamins. Indole-3-acetic acid is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including cyanobacteria [8].

Liberation of extra-metabolites has been found maximum during the stationary phase of growth. The growth regulators reported in rice fields are gibberellins, auxin, cytokinin, ethylene, and abscisic acid by addition of cyanobacterial biofertilizer [14, 18] and their extracts promote overall plant growth [11, 12, 16]. The objectives of the present work is to produced cyanobacteria biomass, total protein and Indole-3-acetic acid during different incubation time using BG-11 broth and evaluated via standard protocols.

MATERIAL AND METHODS**Sample collection**

Six to eight soil samples were collected from agricultural fields around Ratlam district, M.P., India. Composite sample was obtained via mixed all samples together and grinded using mortar and pestle the soil sample was clean to remove plant debris and rock particles.

Isolation of blue green algae

Weigh 10 g soil and serially diluted using a standard dilution technique [1] and BG-11 broth with nitrogen [7]. The soil sample was dissolve in Erlenmeyer conical flask having 95 ml 0.85% saline water and mixed well, mark as 10^{-1} dilution. 10 ml sample was transferred from 10^{-1} dilution to next conical flask containing 90 ml saline water, marks as 10^{-2} dilution. One milliliter sample was transferred to three tubes having 10 ml BG-11 broth and tubes were incubated at 30°C in a continuously illuminated chamber, 4000 lux, for 3 weeks [7].

Identification of blue green algae

Microscopic observation was done by spreading isolated culture on glass slide using forceps

and needles. Culture was covered with glass cover slips and observed under low (10X) and high power (40X) objective lens of compound light microscope. Pure forms of cyanobacteria were identified on the basis of morphological characteristics mentioned in Bergey's Manual of Determinative Bacteriology and Bergey's Manual of Systematic Bacteriology, 2nd ed. Vol. 1 [4, 5].

Biomass Production

Pure culture of *Nostoc* spp. was inoculated in conical flask containing 100 ml BG-11 broth and incubated at 30°C for 10, 15, 20, 25 and 30 days in continuously illuminated chamber, 4000 lux. Biomass was obtained by filtration using Whatman No. 1 filter paper and dry in hot air oven at 50°C for 2 hrs to remove extra moisture then weigh total biomass [7].

Protein production

Nostoc spp. was transferred to conical flask have 100 ml BG-11 broth and incubated at 30°C for 10, 15, 20, 25 and 30 days in continuously illuminated chamber. Culture filtrate was obtained by filtration using Whatman No. 1 filter paper.

Estimation of total protein by Lowry method

Lowry reagents [13] were mix with 0.2 ml culture filtrate and incubated at 37°C for 20 min. in water bath. Color density was recorded at 660 nm using spectrophotometer and compared with standard curve of protein (BSA, 1.0mg/ml).

Indole-3-acetic acid (IAA) production

Selected cyanobacterial isolate i.e. *Nostoc* spp. was transferred to Erlenmeyer conical flask containing 100 ml BG-11 broth. The conical flask was incubated at 30°C for 10, 15, 20, 25 and 30 days in continuous light exposure and culture filter was obtained by filtration using Whatman No.1 filter paper then used for extraction.

Extraction

Firstly set pH of culture filtrate at 2.8 with HCl then IAA was extracted three times with three volumes of ethyl acetate using glass separating funnel and mixed. The extract was evaporated at 37°C using water bath and adjusted pH 7.0 with NaOH.

Estimation of IAA by Salkowski method

3 ml extract was taken in test tube and added 2 ml salkowski reagent [9]. The test tube was incubated at room temperature for 30min. and the color density was recorded at 530 nm using spectrophotometer. The optical density (OD) of sample was compared with standard curve of IAA (500 µg/ml).

RESULTS AND DISCUSSION

The biomass and metabolite estimation of various incubation extract was evaluated using standard protocols and have found following estimations given in Table-1 to 3.

Results obtained to evaluate total biomass are shown in Table-1 and Fig. - 1 that biomass was increased as incubation period increased. Further Table-1 indicates that minimum biomass (40 mg) produced within 10 days; metabolites are utilized by *Nostoc* spp. then cyanobacteria produced more biomass (120 mg) within 15 days and then growth of *Nostoc* spp. was slowly increased. After 25 days growth was enhanced due to available of more growth regulator in medium and produced 300 mg biomass, recorded after 30 days incubation time [7].

Table-2 and Fig.-2 reveals that 195 and 750 µg/ml protein was produced by *Nostoc* spp. within 20 and 30 days incubation time respectively. Fig.- 2 indicates that amount of protein was increased up to 15 days incubation time then decreased due to utilization for their building blocks. The concentration of protein was increased again and reaches maximum as 361 and 750 µg/ml within 25 and 30 days respectively [7].

Results obtained from Table - 3 and Fig. - 3 that IAA produced in culture filtrate of *Nostoc* spp. after 25 and 30 days incubation time that is 1.13 and 4.0 mg/L respectively. Indole-3-acetic acid is a plant growth regulator and its very minute quantity required for their growth. *Nostoc* spp. multiplied in soil and synthesized then secrets extracellular IAA during stationary condition which available in soil for plant growth [11, 12, 16].

Table- 1: Estimation of total biomass of *Nostoc* spp.

S. No.	Incubation days	Biomass (mg)
1	10	40
2	15	120
3	20	160
4	25	180
5	30	300

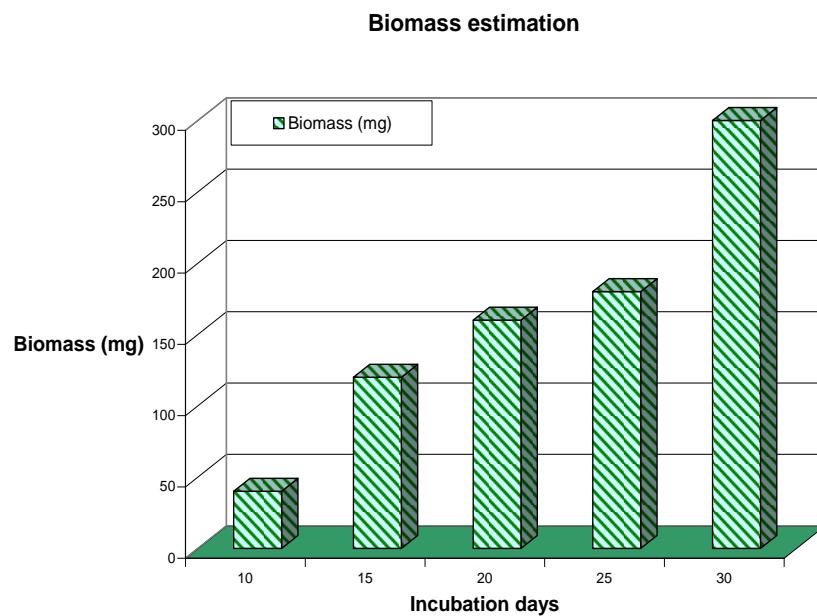


Figure-1: Total biomass estimation of *Nostoc* spp. during different incubation time.

Table-2 Estimation of total protein in culture filtrates of *Nostoc* spp.

S. No.	Incubation days	Total Protein ($\mu\text{g/ml}$)
1	10	466
2	15	581
3	20	195
4	25	361
5	30	750

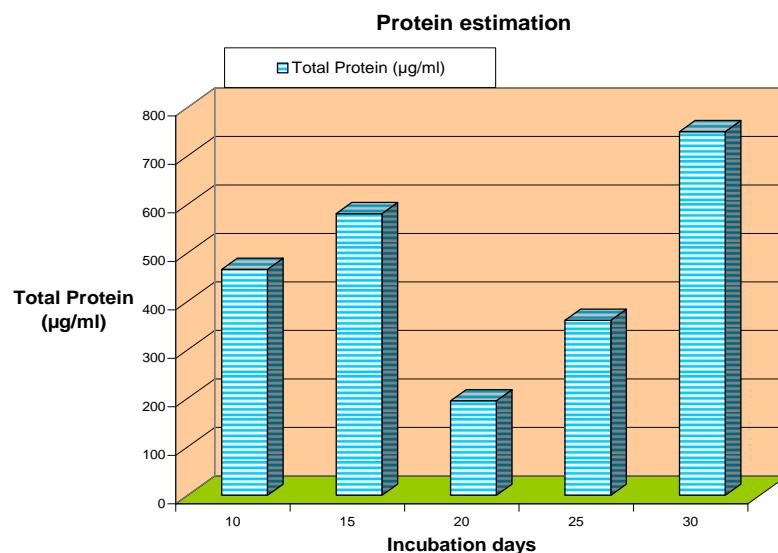
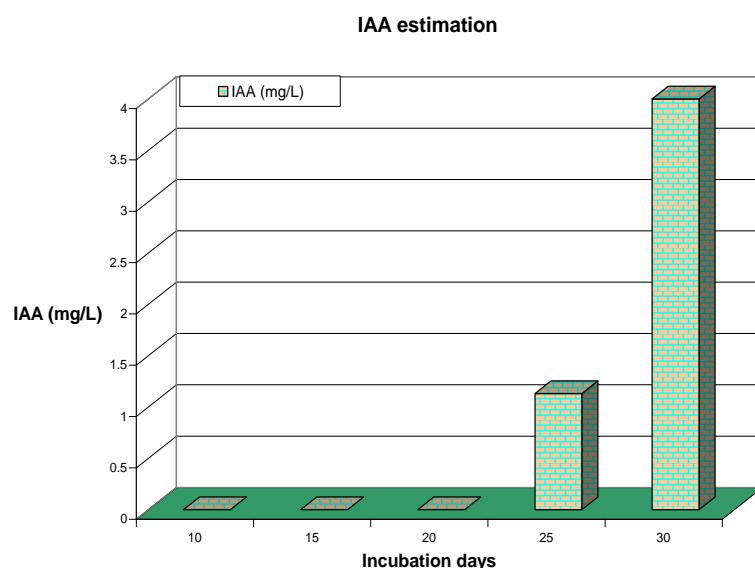


Figure- 2: Total protein estimation in culture filtrates of *Nostoc* spp.

Table-3 Estimation of IAA in culture filtrates of *Nostoc* spp.

S. No.	Incubation days	IAA (mg/L)
1	10	0.0
2	15	0.0
3	20	0.0
4	25	1.13
5	30	4.00

**Figure- 3: Indole-3-acetic acid estimation in culture filtrates of *Nostoc* spp.**

CONCLUSION

By evaluating it was found that amount of biomass and extracellular protein was increased with increasing incubation time but Indole-3-acetic acid was detected after 25 days, it produced in late growth phase and slowly concentration was increased. Both protein and IAA is nitrogenous compound and available in culture filtrate of *Nostoc* spp. Similarly also available in soil after growth of cyanobacteria and improves their fertility as biofertilizer.

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